

We claim:

1. A transfection agent for the non-covalent association with and transport of a heterologous compound into a cell, said transfection agent comprising:

5 a peptide of between about 16 and 30 amino acid residues in length, said peptide comprising:

a hydrophobic domain;

a hydrophilic domain;

optionally a spacer sequence between said domains; and

10 further optionally a functional group conjugated to one or more termini of said peptide.

2. The transfection agent of claim 1 wherein said hydrophobic domain is characterized by a plurality of aromatic amino acids.

15 3. The transfection agent of claim 1 wherein said hydrophilic domain is a cation-rich sequence comprised of at least two lysine residues within a span of seven residues.

4. The transfection agent of claim 2 wherein at least two of said plurality occur in a pair.

20 5. The transfection agent of claim 3 wherein two or more of said at least two lysine residues are adjacent to one another.

6. The transfection agent of claim 2 wherein said plurality is between 3 and 5 inclusive.

25 7. The transfection agent of claim 2 wherein said plurality comprises at least 2 tryptophan residues.

8. The transfection agent of claim 4 comprising two pairs, said pairs separated by 2 amino acids.

9. The transfection agent of claim 8 wherein said 2 amino acids separating said pairs consist of hydrophilic amino acids.

10. The transfection agent of claim 9 wherein said hydrophilic amino acids separating said pairs are Glu and Thr.

11. The transfection agent of claim 1 wherein said peptide is a synthetic peptide.

12. The transfection agent of claim 1, wherein said peptide comprises one or more sequences selected from the group of sequences consisting of SEQ ID NO. 1 through 12.

13. The transfection agent of claim 1, wherein said peptide comprises the sequence: Xaa Xaa Xaa Lys Lys Arg Arg Xaa Xaa Xaa Xaa Xaa Thr Trp Xaa Glu Thr Trp Trp Xaa Xaa Xaa (SEQ ID NO:13), wherein any Xaa can be any amino acid, or can be omitted.

14. The transfection agent of claim 1 wherein said peptide comprises the sequence Lys Xaa Xaa Trp Trp Glu Thr Trp Trp Xaa Xaa Xaa Ser Gln Pro Lys Lys Xaa Arg Lys Xaa (SEQ ID NO:15), wherein any Xaa that is one or two amino acids away from a tryptophan is a hydrophilic amino acid, and wherein an amino acid denoted by Xaa that is three amino acids away from a tryptophan is a hydrophobic amino acid, and wherein an amino acid denoted by X that is positioned at the C-terminus can be any amino acid or can be omitted.

15. The transfection agent of claim 1 wherein said peptide comprises the sequence: Tyr Gly Phe Lys Lys Xaa Arg Arg Pro Trp Thr Trp Trp Glu Thr Trp Trp Thr Glu Xaa (SEQ ID NO:17),

wherein an amino acid denoted by Xaa that is in the hydrophilic domain is a hydrophobic amino acid, and wherein an amino acid denoted by Xaa that is at the C-terminus can be any amino acid or can be omitted.

16. The transfection agent of claim 1, wherein:

- a) at least one of positions eight through thirteen is a proline (Pro), glutamine (Gln), glycine (Gly), tyrosine (Tyr), or serine (Ser) residue;
- b) Xaa at position sixteen is an aromatic hydrophobic amino acid; and
- c) Xaa residues at positions 21 and 22 are preferably hydrophilic amino acids.

17. The transfection agent of claim 13 wherein at least one of said threonine (Thr) residues is instead a tyrosine (Tyr) residue.

18. The transfection agent of claim 1 wherein said optional spacer sequence comprises one or more amino acids selected from the group of amino acids consisting of proline, glycine, tyrosine, serine, glutamine, and non-charged amino acids.

19. The transfection agent of claim 1 wherein said hydrophobic domain comprises the motif Trp-Trp-Xaa-Xaa-Trp of **SEQ ID NO: 18**, wherein the Xaa of said motif is a hydrophilic amino acid, and wherein tyrosine (Tyr) may optionally be substituted for at least one of said tryptophan (Trp).

20. The transfection agent of claim 1 wherein said agent is used to transfect one or more members from the group of compounds consisting of peptides, proteins, antibodies, and derivatives and analogs thereof, and optionally wherein a distinct compound is covalently affixed to said agent and is also transported into said cell.

21. The transfection agent of claim 19 wherein said agent has a transfection efficiency of at least 5% for at least two of said members of said group of compounds.

22. The transfection agent of claim 19 wherein said agent is used to transfect a compound or complex of about 200 kD or less in size.

23. The transfection agent of claim 19 wherein said agent is used to transfect a compound into a living cell, said compound further selected from the group consisting of reporter molecules, molecules that enhance the activity or formation of a cellular or viral polypeptide within a cell, and molecules that inhibit the activity or formation of a cellular or viral polypeptide within a cell.

24. The transfection agent of claim 22 wherein said compound is capable of disrupting the formation of an enzyme that is active as a multimer *in vivo* or *in vitro*.

25. The transfection agent of claim 22 wherein said agent is non-covalently complexed with said compound prior to transfection.

26. The transfection agent of claim 1 that has said one or more covalently attached functional groups, wherein a member from the group consisting of cysteamine, methyl, and alkyl is conjugated to a carboxy terminus of said peptide, and wherein if a group is present on an amino terminus of said peptide, it is an acyl group.

27. The transfection agent of claim 1 wherein said hydrophilic domain comprises the sequence Lys-Arg-Lys, and wherein said spacer sequence comprises at least three amino acid residues of which at least one is a proline or glutamine residue.

28. The transfection agent of claim 1 that is effective to transfect cells of interest using molar ratios of agent:compound to be transfected of between 5:1 and 30:1.

29. The transfection agent of claim 28 used at a working concentration of between about 0.1 uM and 100 uM.

30. The transfection agent of claim 29 used at a molar concentration of between about 1 uM and 20 uM.

31. A commercial transfection kit comprising at least one transfection agent according to claim 1, said kit further comprising one or more components selected from the group consisting of buffers, positive controls, cells to be transfected, phospholipids, and instructions for use; and wherein said agent is supplied either as an aqueous or lyophilized stock.

32. A commercial transfection kit comprising:

one or more peptide transfection agents of between about 16 and 30 amino acid residues in length, said agents characterized by:

(a) a hydrophobic domain;

(b) a hydrophilic domain;

(c) optionally a spacer sequence between said domains; and

(d) optionally one or more covalently attached functional groups selected from the group consisting of stabilizers, couplers, dyes, ligands, enzymatic substrates, and combinations thereof; said kit optionally further comprising:

one or more components selected from the group consisting of buffers, positive controls, cells to be transfected, phospholipids, and instructions for use.

33. The commercial transfection kit of claim 32 wherein said transfection agent is used to promote the cellular internalization of one or more members selected from the group consisting of peptides, proteins, antibodies, and derivatives, conjugates or combinations thereof.

5 34. The commercial transfection kit of claim 33 wherein said agent is non-covalently complexed with one or more of said members preceding transfection.

35. The commercial transfection kit of claim 32 wherein transfection is accomplished using a molar ratio of agent: member of between 5:1 and 30:1.

10 36. The commercial transfection kit of claim 34 wherein transfection is performed using between about 0.1 uM and 100 uM of transfection agent.

37. The commercial transfection kit of claim 35 wherein transfection is performed using  
15 molar concentrations of between about 1 uM and 20 uM of peptide agent.

38. The kit of claim 31 wherein said kit comprises one or more sequences selected from the group of sequences consisting of Seq. I.D. Nos. 1-12.

20 39. A composition of matter comprising a peptide or mixture of peptides consisting essentially of one or more members selected from the group consisting of Seq. I.D. Nos. 1-12 and variant sequences thereof.

25 40. A pharmaceutical composition comprising a transfection agent according to claim 1 or claim 31.

41. The pharmaceutical composition of claim 39 or 40 wherein prior to use said transfection agent is non-covalently complexed with a compound to be delivered to a cell.

42. The pharmaceutical composition of claim 41 wherein said compound comprises a member selected from the group consisting of diagnostic compounds and therapeutic compounds.

43. The pharmaceutical composition of claim 39 or 40 wherein said compound is administered *ex vivo*.

44. The pharmaceutical composition of claim 39 or 40 wherein said compound is administered *in vivo*.

45. The pharmaceutical composition of claim 41 wherein said compound is a therapeutic compound that is effective to treat one or more afflictions selected from the group consisting of cancer and infectious diseases.

46. The pharmaceutical composition of claim 41 wherein said compound is p53.

47. The pharmaceutical composition of claim 41 wherein said composition is used to deliver a peptide or inhibitor that disrupts the activity of an enzyme.

48. The pharmaceutical composition of claim 47 wherein said compound targets a cancerous cell.

49. The pharmaceutical composition of claim 47 wherein said compound targets a pathogen-infected cell.

50 A method of delivering a polypeptide compound to a target cell comprising:

providing a non-covalent complex of a peptide transfection agent and compound to be delivered, wherein said peptide transfection agent is present in a greater molar amount than said compound in said complex; and

5 contacting a target cell with said complex under one or more environmental conditions; wherein said peptide transfection agent comprises a peptide between about 16 and 30 amino acid residues in length, and further comprises:

(a) a hydrophobic domain;

(b) a hydrophilic domain;

10 (c) optionally a spacer sequence between said domains; and

(d) further optionally one or more covalently attached functional groups selected from the group consisting of stabilizers, couplers, dyes, ligands, enzymatic substrates, and combinations thereof.

15 51 The method of claim 51 wherein said hydrophobic domain comprises a plurality of aromatic amino acids at least two of which are no greater than 3 amino acid residues apart, wherein at least two of said plurality are tryptophan, wherein said hydrophilic domain comprises or derives from a nuclear localization signal, and wherein said optional spacer sequence between said domains comprises between 2 and 8 amino acid residues inclusive, at least one of which is a proline or analog thereof.

20 52. The method of claim 51 wherein at least two of said aromatic amino acids occur in a pair.

53. The method of claim 51 wherein said plurality comprises 3 to 5 aromatic amino acids.

25 54. The method of claim 52 wherein at least one pair is present, said pair consisting of 2 tryptophan residues.



55. The method of claim 51 wherein if multiple pairs occur they are separated by at least 2 amino acids.

56. The method of claim 55 wherein said at least 2 amino acids separating said pairs consist of hydrophilic amino acids.

57. The method of claim 56 wherein said at least two hydrophilic amino acids separating said pairs are Glu and Thr.

58. The method of claim 50 wherein said peptide is a synthetic peptide.

59. The method of any of claims 51-60 wherein said polypeptide compound is selected from the group consisting of peptides, proteins, antibodies, and derivatives and analogs thereof.

60. The method of claim 59 wherein said compound is between about 10 kD and 200 kD in size.

61. The method of claim 59 wherein said compound is further selected from the group consisting of antibodies and derivatives thereof.

62. The method of claim 50 wherein said complex comprises a molar ratio of peptide transfection agent :compound of at least 5:1.

63. The method of claim 59 wherein said complex comprises a molar ratio of peptide transfection agent:compound of at least 5:1.

64. The method of claim 51 or 59 wherein said transfection is performed using between about 0.1  $\mu$ M and 100  $\mu$ M of vector.

65. The method of claim 64 wherein transfection is performed using between about 1  $\mu$ M and 20  $\mu$ M of peptide agent.

66. The method of claim 50 wherein said peptide vector comprises one or more members selected from the group consisting of Seq. I/D. Nos. 1-12, and variants thereof.

67. The method of claim 50 wherein said compound that is transfected is part of a library of compounds.

68. The method of claim 67 further comprising transfecting said library or a sublibrary thereof into a cell or population of cells and assaying for an effect on said cell.

69. The method of claim 68 wherein said library is a peptide or protein library.

70. The method of claim 69 wherein a peptide is identified from said assay.

71. The method of claim 70 wherein said peptide identified from said assay has a cellular analog within said transfected cell, said analog encoded by a gene, and said peptide is used to isolate said gene.

72. A method of identifying a peptide potentially useful as a transfection agent for the non-covalent association with, and delivery of a polypeptide compound to a target cell, said method comprising:

(a) providing as a standard one or more of a peptide and a cationic lipid each of which is known to be useful as a transfection agent for the non-covalent association with, and delivery of, a polypeptide compound to a target cell;

(b) providing a test peptide having a sequence different than said standard, said test peptide comprising a peptide of between about 16 and 30 amino acid residues in length, said peptide having a hydrophobic domain and optionally further including a hydrophilic cation-rich domain;

(c) assaying for comparative effect said one or more standards against said test peptide under one or more environmental conditions; and

(d) comparing the relative data achieved to thereby identify a test peptide that is potentially useful as a transfection agent for the non-covalent association with, and delivery of, a compound to a target cell.

73. The method of claim 72 wherein said assaying comprises the performing of one or more procedures selected from the group of assays consisting of gel retardation assays, affinity binding assays, circular dichroism measurements, nmr, fluorescence quenching, FTIR spectroscopy, transfection efficiency into a target cell whether or not complexed with a compound of interest, addressing ability within a cell, toxicity to a target cell, ability to transport said compounds to a subcellular organelle, ability to transport differently sized compounds, ability to transport differently charged compounds, ability to protect or enhance the stability of said compound or compounds from degradation, and ability to adopt a structured conformational state.

74. The method of claim 72 or 73 wherein said compound is selected from the group of compounds consisting of peptides, proteins, antibodies, and derivatives and analogs thereof.

75. The method of claim 72 or 73 wherein said compound is between about 10 kD and 200 kD.

For information